3-1-2018

Physiological responses to heat stress in two genetically distinct chicken inbred lines

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Abstract

High ambient temperature is one of the most important environmental factors negatively impacting poultry production and health. Genetics is an important contributor in mitigating the stress response to heat. Two genetically distinct highly inbred lines of similar body size (Leghorn and Fayoumi) were characterized for phenotypic differences in response to heat. At 14 days of age, birds were exposed to 38°C with 50% humidity for 4 hours, then 35°C until the conclusion of the experiment. Non-treated individuals were kept at 29.4°C for the first week and then 25°C throughout the experiment. Birds in the heat-stress group were inoculated at day (d) 21 with Newcastle disease virus (NDV) La Sota strain to investigate the effects of heat stress and NDV infection. Thirteen blood parameters were measured using the iSTAT blood analyzer at three stages: 4 h, 6 d, and 9 d post heat-stress treatment, representing acute heat (AH) exposure, chronic heat (CH1) exposure, and chronic heat exposure after virus infection (CH2), respectively. Most blood parameters were significantly changed with heat stress in Leghorns at AH and in Fayoumis at CH1 and CH2. The Leghorn line had significant acute responses with disrupted acid-base balance and metabolic disorders. The heat-resilient Fayoumis maintained a relatively well-balanced acid-base balance. The current study provides the comprehensive profile of biomarker signatures in blood associated with heat tolerance and suggests that PO2, TCO2, HCO3, and base excess can be served as potential biomarkers that can be used to genetically improve heat tolerance in poultry.

Keywords

heat stress, physiological response, chicken, inbred line

Disciplines

Agriculture | Animal Sciences | Genetics | Poultry or Avian Science

Comments

This article is published as Wang, Y., P. Saelao, K. Chanthavixay, R. Gallardo, D. Bunn, S. J. Lamont, J. M. Dekkers, T. Kelly, and H. Zhou. "Physiological responses to heat stress in two genetically distinct chicken inbred lines." *Poultry Science* 97, no. 3 (2018): 770-780. DOI: 10.3382/ps/pex363. Posted with permission.

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Physiological responses to heat stress in two genetically distinct chicken inbred lines

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ABSTRACT High ambient temperature is one of the most important environmental factors negatively impacting poultry production and health. Genetics is an important contributor in mitigating the stress response to heat. Two genetically distinct highly inbred lines of similar body size (Leghorn and Fayoumi) were characterized for phenotypic differences in response to heat. At 14 days of age, birds were exposed to 38°C with 50% humidity for 4 hours, then 35°C until the conclusion of the experiment. Non-treated individuals were kept at 29.4°C for the first week and then 25°C throughout the experiment. Birds in the heat-stress group were inoculated at day (d) 21 with Newcastle disease virus (NDV) La Sota strain to investigate the effects of heat stress and NDV infection. Thirteen blood parameters were measured using the iSTAT blood analyzer at three stages: 4 h, 6 d, and 9 d post heat-stress treatment, representing acute heat (AH) exposure, chronic heat (CH1) exposure, and chronic heat exposure after virus infection (CH2), respectively. Most blood parameters were significantly changed with heat stress in Leghorns at AH and in Fayoumis at CH1 and CH2. The Leghorn line had significant acute responses with disrupted acid-base balance and metabolic disorders. The heat-resilient Fayoumis maintained a relatively well-balanced acid-base balance. The current study provides the comprehensive profile of biomarker signatures in blood associated with heat tolerance and suggests that PO_2, TCO_2, HCO_3, and base excess can be served as potential biomarkers that can be used to genetically improve heat tolerance in poultry.

Key words: heat stress, physiological response, chicken, inbred line

2018 Poultry Science 97:770–780
http://dx.doi.org/10.3382/ps/pex363

INTRODUCTION

Similar to other homoeothermic animals, chickens maintain a constant body temperature over a wide range of ambient temperatures (Deeb and Cahaner, 1999). However, with the global climate change, poultry production is increasingly impacted by high ambient temperatures, especially in developing countries where poultry are mainly reared in environments without temperature control. Once ambient temperatures exceed the tolerable range, birds have difficulties with maintaining their homeostatic body temperature, which is classified as "heat stress" (Lara and Rostagno, 2013). Heat stress results in physiological changes, and subsequent negative effects on production performance in poultry.

Heat stress is one of the most important environmentally influenced factors that can negatively affect poultry production and health (Ayo et al., 2011). With heat stress, laying hens typically suffer from significant reductions in production traits such as egg weight, shell thickness, and rate of production (Wolfenson et al., 2001). Fast-growing broilers maintain higher internal body temperatures than layers, and high mortality rates, low growth rate, and reduced meat quality and body weight have been observed under heat stress (Muiruri and Harrison, 1991). Heat stress also affects the immune response in poultry. Immunosuppression has been observed in both broilers and layers during exposure to a heat stress environment (Padgett and Glaser, 2003). Reduced lymphoid organ weight and lower levels of circulating antibodies, including specific IgM and IgY, were detected in heat-stressed birds compared to non-treated birds (Felver-Gant, 2012). Further, antibody response and phagocytosis of macrophages were repressed in chickens under heat stress (Niu et al., 2009).

Received August 14, 2017.
Accepted November 1, 2017.
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Heat stress also affects physiological parameters including chemistry and blood gas parameters in animals and the responses vary depending on the time and degree of thermal challenge (Boddicker et al., 2014). Obtaining accurate and reliable measurements of these parameters used to present a great challenge in animal farming until the recent development of a point of care, portable blood analyzer, i.e., the i-STAT (Abbott Laboratories, San Diego, CA) handheld blood analyzer (Martin et al., 2010). Physiological parameters associated with host response to heat stress can be immediately measured, minimizing alterations in the blood-gas and electrolyte values as a result of sample handling and transportation (Kaneko et al., 2008). The i-STAT has been used to assess blood clinical chemistries in chickens and reference ranges have been established for broiler breeders (Martin et al., 2010). Depending on the purpose, different i-STAT cartridges can be selected. In our study, the CG8+ cartridge was used to evaluate heat-stress-related parameters in blood.

Genetics plays a significant role in affecting host response to heat stress in poultry (Felver-Gant, 2012). The divergent selection in Leghorns has resulted in chicken lines with different levels of heat tolerance (Wilson et al., 1975). In addition, fast-growing broilers were more sensitive to heat stress than slow-growing broiler strains (Yunis and Cahaner, 1999). Genetic resistance to high ambient temperatures in chickens can be inherited from their ancestors (Lu et al., 2007). Indigenous chicken breeds in tropical areas have been documented to have higher heat stress tolerance compared to other breeds (Soleimani and Zulkifli, 2010).

Two genetically distinct highly inbred chicken lines (Leghorn GB2 and Fayoumi M43) maintained at Iowa State University, whose inbreeding coefficients were more than 99.99% (Zhou and Lamont, 1999), were used in the current study. Thus, genetic variations within the line are negligible. The Fayoumis were originally imported from Egypt and they had undergone natural selection for heat tolerance (Lamont et al., 2014). Based on previous reports, Fayoumi birds are more resistant to both disease and heat stress (Bjorkquist et al., 2015; Wang et al., 2014). A broiler (heat-susceptible) × Fayoumi (heat-resistant) advanced intercross line (**AIL**) was used to fine-map quantitative trait loci (**QTL**) using the 600 K Affymetrix chicken single nucleotide polymorphism (**SNP**) array to investigate genetic influence of host response to heat stress in chickens (Van Goor et al., 2015). Several QTL regions had been identified including one major QTL for breast muscle yield under heat stress which accounted for more than 24% of the genetic variation in this study. Thirty-five QTLs were identified to be associated with the response to heat stress. In addition, association of physiological changes on blood components and genomic regions were identified on the same chicken AIL line, which may serve as markers for genetic selection to enhance heat tolerance in poultry (Van Goor et al., 2016). Most recently, the Fayoumi and broiler breeds were also used to study host transcriptome response to heat and Lipopolysaccharide (**LPS**) stimulation using RNA-seq (Van Goor et al., 2017) and genes and signal pathways associated with heat stress and LPS identified can be served as additional biomarkers for heat tolerance. Based on these studies, some heat tolerant favorable alleles might be fixed in Fayoumi breed, which can be utilized for genetic selection. We hypothesize that Leghorns are relatively heat susceptible while Fayoumis are more heat tolerant. The current study is a part of a US Agency for International Development sponsored program (Feed the Future Innovation Lab for Genomics to Improve Poultry) to improve food security in Africa by enhancing resistance to Newcastle disease and heat stress in chickens (http//:gip.ucdavis.edu). Our major focus in the current study was to characterize physiological responses to heat stress in these two diverse highly inbred lines and develop candidate diagnostic biomarkers for potential genetic selection to improve heat tolerance in poultry.

MATERIALS AND METHODS

Experimental Populations

One hundred and eleven birds, 55 Leghorns and 56 Fayoumis, were housed in two temperature and humidity-controlled isolators, and birds were provided with ad libitum access to food and water. On day (d) 1 of age, 30 Leghorn and 31 Fayoumi birds were randomly chosen as the treatment groups and housed in one isolator and the rest of birds were used as the non-treated groups in another isolator. The two lines were mixed in each isolator. From 14 days of age to the end of the experiment (41 days of age), the heat-treated groups were exposed to continuous heat stress of 38°C for the first 4 hours, and then decreased to 35°C, while the non-treated groups were maintained at 29.4°C for the first week and 25°C throughout the whole experiment. On d 21, birds in the heat-treated groups were inoculated with 10^7 EID_{50} Newcastle Disease virus (**NDV**) La Sota strain through both ocular and nasal passages (50 μL per nostril and eye). The animal experiment was performed according to the guidelines approved by the Institutional Animal Care and Use Committee, University of California, Davis (IACUC #17853).

Blood Parameters Measurements

Blood gases and chemistry parameters were measured in all birds at d14 (4 hour post-heat-stress treatment, acute phase (**AH**)), d20 (6 days post-treatment, chronic phase 1 (**CH1**)) and d23 (9 days post-treatment, chronic phase 2 (**CH2**)), respectively. Meanwhile, six birds per line per group were euthanized for tissue collection (RNA-seq analysis on separate study) at d14 and d23. Approximately 1 mL of blood was collected into a heparinized syringe (2,000 unit/mL

heparin in PBS, Sigma-Aldrich, St. Louis, MO) for each bird, and then blood samples were analyzed immediately using an i-STAT Portable Blood Analyzer. The i-STAT cartridge (CG8+) measured 13 different parameters including four chemistry/electrolyte parameters (concentrations of sodium (Na^+), potassium (K^+), ionized calcium (Ca^{2+}), and glucose (**Glu**)); seven blood gas parameters (blood pH, carbon dioxide partial pressure (**PCO_2**), oxygen partial pressure (**PO_2**), total carbon dioxide (**TCO_2**), bicarbonate (HCO_3), BE, and oxygen situation (**sO_2**)), and two hematologic parameters: hematocrit (**Hct** or packed cell volume (**PCV**)), and hemoglobin (**Hb**). TCO_2 measures the total carbon dioxide and is calculated from pH and PCO_2. Measurement of TCO_2 is useful to assess HCO_3 concentration (Ungerer et al., 1990). Base excess (**BE**) can be estimated from HCO_3 and pH.

Statistical Analysis

The i-STAT parameters were analyzed using the following linear model for each time point separately in JMP 13 software (SAS Institute, 2016), since population size continued to decrease due to tissue collections at each time point.

$$Y = \mu + T + L + T \times L + e$$

Where Y is the dependent variable of various parameters, μ is the population mean of the measurements, T is the fixed effect associated with the heat stress treatment, L is the fixed effect associated with the genetic line, $T \times L$ is the interaction effect of treatment, and line and e is the random error effect. All measurements in this study were represented as least square mean ±SE. $P < 0.05$ was considered statistically significant. All figures were generated by Prism 6 GraphPad software (La Jolla, CA).

RESULTS

Blood Parameter Measurements

Lease square means and P values for each factor were listed in Table 1 for all blood parameters of both genetic lines at each time point. Physiological responses to heat stress between the two genetic lines and within each genetic line were compared based on the blood parameter measurements. At the AH stage, six (K^+, iCa^{2+}, pH, PCO_2, PO_2, and sO_2) out of thirteen parameters had significant $T \times L$ interactions ($P < 0.05$). For the rest seven parameters, all main genetic line effects were significant except Glu. While only two parameters (Na^+ and BE) were significant on main treatment effects. At the CH1 stage, seven (K^+, Glu, PCO_2, TCO_2, HCO_3, PO_2, and BE) out of thirteen parameters had significant $T \times L$ interactions ($P < 0.05$). Significant main treatment effects were identified on four parameters (pH, sO_2, Hct, and Hb) and no main genetic line effects were observed. At the CH2 stage with 2 days post inoculation (**DPI**) NDV inoculation, five parameters (Glu, TCO_2, HCO_3, PO_2, and sO_2) had significant $T \times L$ interactions ($P < 0.05$). The main treatment effect was identified on pH and BE; and there were three parameters (Na^+, pH, and BE) had main genetic line effects. For blood parameters having significant $T \times L$ effects, line effects in treated and non-treated groups and treatment effects within genetic lines were further analyzed.

Effects of Heat Stress between Genetic Lines

Without heat stress, the two genetic lines generally had 7 out of 13 parameters significantly difference due to their genetic background which were iCa^{2+}, K^+, PCO_2, TCO_2, HCO_3, BE, and PO_2 on d14 (Figure 1); 6 out of 13 parameters (K^+, PCO_2, TCO_2, HCO_3, BE, and PO_2) on d 20 (Figure 2); and 6 out of 13 parameters (Na^+, pH, TCO_2, HCO_3, PO_2, and BE) on d 23 (Figure 3). Across all time points, Leghorn birds had significantly higher PO_2 and lower TCO_2, HCO_3, and BE than Fayoumi birds. They also had higher K^+ and lower levels of PCO_2 than Fayoumis on both d14 and d20 and higher pH and lower Na^+ levels than Fayoumis on d23.

With heat treatment, more parameters (8 out of 13) were significantly different between the two genetic lines at the AH stage, which were Na^+, K^+, pH, PCO_2, sO_2, BE, Hct, and Hb (Figure 4). One week later at the CH1 stage, there were no differences in parameters between the Fayoumi and Leghorn lines. At the CH2 stage (after NDV infection), Leghorn birds had higher Glu and PCO_2 and lower Na, sO2, pH, and sO_2 levels than Fayoumis (Figure 5).

Effects of Heat Stress within Genetic Lines

At the AH stage Leghorn birds had more vigorous physiologic responses than Fayoumis with acute heat stress. Comparing with the non-treated groups, there were 7 parameters changed in the Leghorns upon heat stress, which were Na^+, K^+, iCa^{2+}, Glu, HCO_3, PO_2, and sO_2 (Figure 6); while only 3 parameters (pH, PCO_2 and sO_2) were significantly changed in Fayoumi birds (Figure 7). Heat stressed Leghorns had significantly lower Na^+, K^+ and higher iCa^{2+}, Glu than the non-treated birds (Figure 6). There were no differences in blood chemistry/electrolytes in Fayoumi birds at the AH stage (Figure 7). For the five CO_2 associated parameters, the HCO_3 level in the heat stress Leghorns was the only one increased comparing with non-treated leghorns and there was no change in the rest four parameters (Figure 6). Higher pH and lower PCO_2 levels were observed in heat stressed Fayoumis than non-treated ones (Figure 7). For the two oxygen associated

Table 1. Lease square means and *P* values of blood parameters with Heat stress treatment.

		LS mean ± SE				*P* values		
	Blood parameter	LC	LT	FC	FT	Trt	Line	Line*Trt
Acute Heat	Na^+ (mmol/L)	142.21 ± 0.31^{A}	140.8 ± 0.27^{B}	142.78 ± 0.31^{A}	142.48 ± 0.28^{A}	**0.0045**	**0.0002**	0.0619
	K^+ (mmol/L)	4.90 ± 0.13^{A}	4.31 ± 0.11^{C}	4.52 ± 0.13^{BC}	4.66 ± 0.12^{AB}	0.0702	0.90	**0.0036**
	iCa^{2+} (mmol/L)	0.89 ± 0.05^{B}	1.09 ± 0.04^{A}	1.03 ± 0.05^{A}	1.03 ± 0.04^{A}	**0.0347**	0.3557	**0.0270**
	Glu (mg/dL)	218.96 ± 6.08^{B}	238.50 ± 5.44^{A}	222.61 ± 6.22^{AB}	225.38 ± 5.54^{AB}	0.0585	0.4185	0.1534
	pH	7.47 ± 0.015^{B}	7.49 ± 0.017^{B}	7.48 ± 0.017^{B}	7.59 ± 0.015^{A}	**0.0002**	**0.0035**	**0.0047**
	PCO_2 (mmHg)	25.55 ± 1.02^{BC}	26.70 ± 0.93^{B}	30.35 ± 1.04^{A}	23.46 ± 0.93^{C}	**0.0043**	0.4272	**0.0001**
	TCO_2(mmol/L)	19.50 ± 0.65^{C}	21.21 ± 0.59^{BC}	23.26 ± 0.66^{A}	22.62 ± 0.59^{AB}	0.3941	**0.0001**	0.0626
	HCO (mmol/L)	18.68 ± 0.64^{C}	20.46 ± 0.58^{B}	22.30 ± 0.65^{A}	21.95 ± 0.58^{AB}	0.2450	**0.0001**	0.0842
	PO_2 (mmHg)	61.38 ± 1.38^{A}	55.37 ± 1.24^{B}	55.83 ± 1.14^{B}	56.45 ± 1.26^{B}	**0.0447**	0.0948	**0.0139**
	sO_2 (%)	92.54 ± 0.73^{AB}	90.21 ± 0.66^{C}	90.57 ± 0.75^{BC}	93.21 ± 0.66^{A}	0.8273	0.4675	**0.0006**
	BE (mmol/L)	− 4.88 ± 0.77^{C}	− 2.90 ± 0.70^{BC}	− 1.17 ± 0.78^{AB}	0.21 ± 0.70^{A}	**0.0250**	**0.0001**	0.6866
	Hct (%PCV)	16.77 ± 0.69^{AB}	18.45 ± 0.61^{A}	16.39 ± 0.68^{B}	15.90 ± 0.61^{B}	0.3640	**0.0257**	0.0969
	Hb (g/dL)	5.70 ± 0.24^{AB}	6.28 ± 0.21^{A}	5.56 ± 0.23^{B}	5.40 ± 0.21^{B}	0.3378	**0.0218**	0.0968
Chronic Heat 1	Na^+ (mmol/L)	142.78 ± 0.38^{B}	143.18 ± 0.39^{AB}	143.53 ± 0.42^{AB}	143.79 ± 0.33^{A}	0.3925	0.0770	0.8547
	K^+ (mmol/L)	4.90 ± 0.11^{A}	4.58 ± 0.11^{BC}	4.43 ± 0.12^{C}	4.83 ± 0.09^{AB}	0.7019	0.3176	**0.0013**
	iCa^{2+} (mmol/L)	0.88 ± 0.06^{A}	0.86 ± 0.06^{A}	1.00 ± 0.06^{A}	0.91 ± 0.05^{A}	0.3240	0.1301	0.5635
	Glu (mg/dL)	201.44 ± 6.92^{A}	215.18 ± 7.12^{A}	216.20 ± 7.58^{A}	201.00 ± 5.99^{A}	0.9159	0.9667	**0.0403**
	pH	7.41 ± 0.02^{B}	7.47 ± 0.02^{AB}	7.44 ± 0.02^{B}	7.50 ± 0.02^{A}	**0.0076**	0.1313	0.9783
	PCO_2 (mmHg)	26.93 ± 1.26^{B}	24.24 ± 1.29^{BC}	31.34 ± 1.43^{A}	23.35 ± 1.09^{C}	**0.0001**	0.1725	**0.0410**
	TCO_2(mmol/L)	17.78 ± 0.71^{B}	18.24 ± 0.73^{B}	22.14 ± 0.81^{A}	19.17 ± 0.62^{B}	0.0858	**0.0005**	**0.0203**
	HCO (mmol/L)	16.98 ± 0.70^{B}	17.52 ± 0.73^{B}	21.26 ± 0.80^{A}	18.30 ± 0.61^{B}	0.0943	**0.0007**	**0.0167**
	PO_2 (mmHg)	59.17 ± 1.90^{A}	59.29 ± 1.95^{A}	51.07 ± 2.08^{B}	60.17 ± 1.64^{A}	**0.0176**	0.0610	**0.0208**
	sO_2 (%)	89.61 ± 0.92^{B}	92.35 ± 0.95^{A}	87.14 ± 1.04^{B}	92.63 ± 0.80^{A}	**0.0001**	0.2127	0.1460
	BE (mmol/L)	− 7.67 ± 0.86^{C}	− 6.12 ± 0.88^{BC}	− 2.93 ± 0.97^{A}	− 4.83 ± 0.74^{AB}	0.8377	**0.0009**	**0.0499**
	Hct (%PCV)	21.00 ± 0.71^{A}	16.63 ± 0.74^{B}	20.53 ± 0.76^{A}	17.13 ± 0.60^{B}	**0.0000**	0.9812	0.4958
	Hb (g/dL)	7.14 ± 0.24^{A}	5.66 ± 0.25^{B}	6.99 ± 0.26^{A}	5.85 ± 0.21^{B}	**0.0000**	0.9176	0.4756
Chronic Heat 2 (+ NDV 2 DPI)	Na^+ (mmol/L)	143.05 ± 0.31^{B}	142.87 ± 0.29^{B}	144.06 ± 0.32^{A}	144.21 ± 0.28^{A}	0.9601	**0.0021**	0.5791
	K^+ (mmol/L)	4.72 ± 0.11^{A}	4.93 ± 0.10^{A}	4.93 ± 0.11^{A}	4.94 ± 0.10^{A}	0.2829	0.3139	0.3266
	iCa^{2+} (mmol/L)	0.84 ± 0.05^{B}	0.98 ± 0.04^{A}	0.95 ± 0.05A^{B}	0.95 ± 0.04A^{B}	0.1403	0.4094	0.1395
	Glu (mg/dL)	184.58 ± 5.91^{C}	220.09 ± 5.37^{A}	197.06 ± 6.07^{BC}	200.71 ± 5.26^{B}	**0.0009**	0.5440	**0.0062**
	pH	7.42 ± 0.02^{B}	7.37 ± 0.01^{C}	7.49 ± 0.02^{A}	7.45 ± 0.01^{B}	**0.0025**	**0.0000**	0.9765
	PCO_2 (mmHg)	27.04 ± 1.09^{AB}	28.47 ± 0.99^{A}	26.85 ± 1.12^{AB}	25.22 ± 0.97^{B}	0.9265	0.1033	0.1471
	TCO_2(mmol/L)	18.16 ± 0.61^{B}	17.39 ± 0.56^{B}	21.39 ± 0.63^{A}	18.08 ± 0.55^{B}	**0.0009**	**0.0013**	**0.0338**
	HCO (mmol/L)	17.36 ± 0.59^{B}	16.56 ± 0.53^{B}	20.47 ± 0.60^{A}	17.16 ± 0.52^{B}	**0.0005**	**0.0015**	**0.0295**
	PO_2 (mmHg)	57.89 ± 1.37^{A}	54.43 ± 1.24^{AB}	51.11 ± 1.41^{B}	54.88 ± 1.22^{A}	0.9081	**0.0180**	**0.0073**
	sO_2 (%)	89.63 ± 0.90^{A}	87.00 ± 0.82^{B}	88.83 ± 0.92^{AB}	89.75 ± 0.80^{A}	0.3224	0.2605	**0.0426**
	BE (mmol/L)	− 7.16 ± 0.70^{BC}	− 8.74 ± 0.64^{C}	− 2.94 ± 0.72^{A}	− 6.79 ± 0.62^{B}	**0.0001**	**0.0000**	0.0954
	Hct (%PCV)	17.06 ± 0.64^{A}	16.17 ± 0.56^{A}	16.44 ± 0.64^{A}	17.17 ± 0.55^{A}	0.8943	0.7505	0.1838
	Hb (g/dL)	5.89 ± 0.21^{A}	5.49 ± 0.19^{A}	5.59 ± 0.21^{A}	5.81 ± 0.18^{A}	0.6512	0.9686	0.1171

Note: LC: Leghorn non-treated group; LT: Leghorn treated group; FC: Fayoumi non-treated group; FT: Fayoumi treated group; Trt: treatment effects; Line: line effects; Line*Trt: line and treatment interaction effects; Different superscript letters within row represent significant differences ($P < 0.05$).

parameters PO_2 and sO_2, Leghorns had significantly lower PO_2 and sO_2 with heat stress treatment (Figure 6), while heat stressed Fayoumis had higher sO_2 levels at AH stage (Figure 7). For the two hematology parameters, Hct and Hb, there were no differences in Hct and Hb levels between heat stressed and non-treated groups of both genetic lines (Figure 6 and 7).

At the CH Stages At the CH1 stage, between heat stressed and non-treated birds, only 4 parameters (K^+, sO_2, Hct, and Hb) were significantly changed in Leghorn birds (Figure 8), compared to 9 parameters (K^+, pH, PCO_2, TCO_2, HCO_3, PO_2, sO_2, Hct, and Hb) changed responding to heat stress in Fayoumis (Figure 9). At the CH2 stage, which was 2 DPI with NDV, Leghorn birds had significant changes in iCa^{2+}, Glu, pH, and sO2 (Figure 10) and Fayoumi birds had more blood gas parameters changed including pH, TCO_2, HCO_3 PO_2, and BE comparing with non-treated birds (Figure 11).

At the CH1 stage, K^+ was lower in the heat stressed Leghorns, while higher in heat stressed Fayoumis compared to non-treated birds (Figures 8 and 9). At the CH2 stage, heat stressed Leghorn birds kept higher levels of iCa^{2+} and Glu compared to non-treated Leghorns (Figure 10), however, there were no significant changes in parameters between heat stressed and non-treated Fayoumis during this stage (Figure 11). With the exception of a lower pH level at the CH2 stage, CO_2 associated blood gas parameters were similar between heat stressed and non-treated Leghorns at both CH1 and CH2 stages (Figures 8 and 10). However, heat stressed Fayoumis kept a higher pH level than non-treated ones at CH1 and the pH level went down to lower than non-treated Fayoumis at CH2 (Figures 9 and 11). Consequently, in Fayoumis, heat stressed birds had significantly lower PCO_2, TCO_2, and HCO_3 levels at the CH1 stage (Figure 9) and much lower levels of TCO_2, HCO_3, and BE at the CH2 stage (Figure 11). The difference

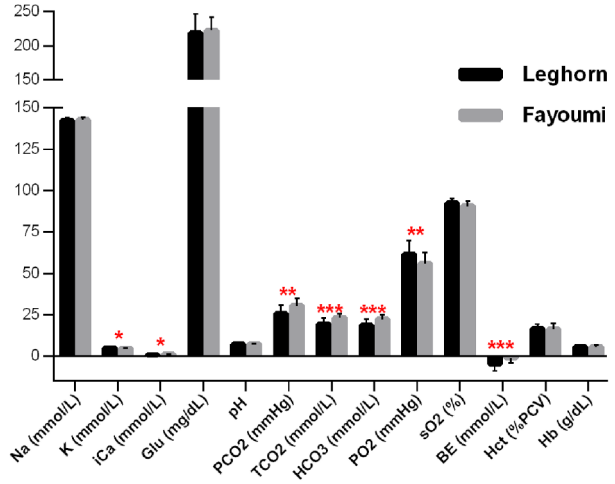

Figure 1. Blood parameter differences between non-treated genetic lines at 14 days of age. Blood parameter differences between non-treated genetic lines at day 14. *indicated significant pairwise difference between lines ($P < 0.05$) **; indicated $P < 0.01$, and ***indicated $P < 0.001$.

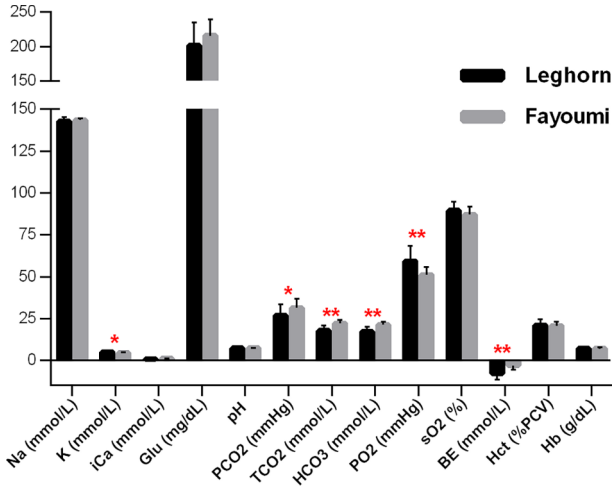

Figure 2. Blood parameter differences between non-treated genetic lines at 20 days of age. Blood parameter differences between non-treated genetic lines at day 20. *indicated significant pairwise difference between lines ($P < 0.05$); **indicated $P < 0.01$.

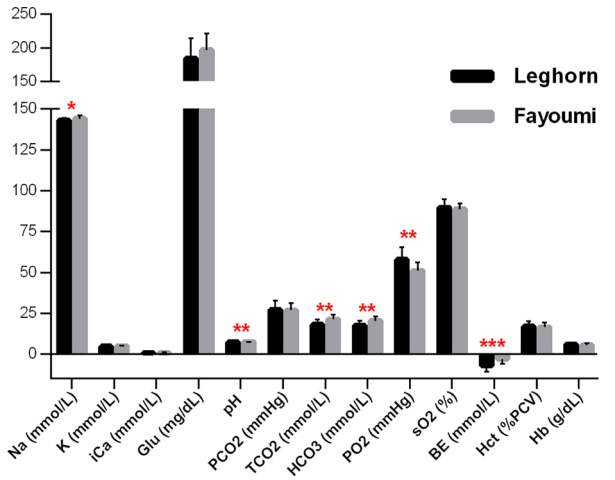

Figure 3. Blood parameter differences between non-treated genetic lines at 23 days of age. Blood parameter differences between non-treated genetic lines at day 23. *indicated significant pairwise difference between lines ($P < 0.05$); **indicated $P < 0.01$, and ***indicated $P < 0.001$.

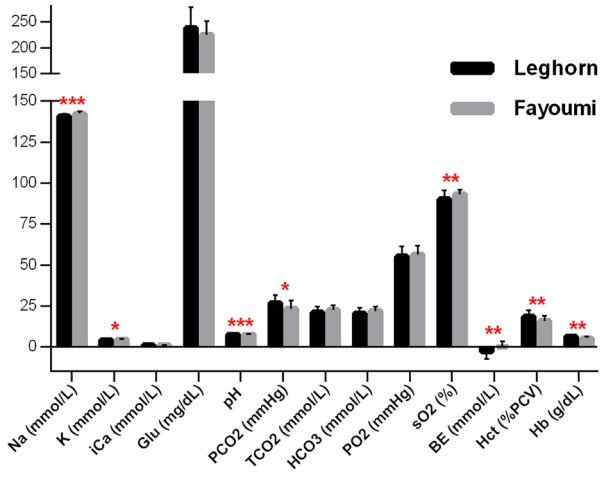

Figure 4. Blood parameter differences between genetic lines at the acute heat stress stage. Blood parameter differences between heat-stress treated genetic lines at the acute heat stage. *indicated significant pairwise difference between lines ($P < 0.05$); **indicated $P < 0.01$, and ***indicated $P < 0.001$.

of the oxygen related parameters was only identified on sO_2 in Leghorns at both CH1 and CH2 stage, in which the sO_2 levels of heat stressed Leghorns were higher than non-treated birds at the CH1 stage (Figure 8) and lower at the CH2 stage (Figure 10). However, heat stressed Fayoumis had higher PO_2 and sO_2 at the CH1 stage (Figure 9) and higher PO_2 at the CH_2 stage (Figure 11). The two genetic lines responded similarly at CH stages on hematology parameters. At the CH1 stage, Hct and Hb were significantly lower in heat stressed birds compared to non-treated birds observed in both lines (Figures 8 and 9). There were no differences in hematologic parameters between heat stress and non-treated birds at the CH2 stage for both genetic lines (Figures 10 and 11).

DISCUSSION

Genetic background plays important roles in heat stress resistance in poultry (Lara and Rostagno, 2013). Different chicken breeds or strains have varied tolerance to heat stress (Deeb and Cahaner, 1999; Yalcin et al., 2001; Cahaner et al., 2008; Islam and Nishibori, 2009). Generally, native or indigenous chicken breeds in the tropical areas have better potential to adapt to changes in ambient temperatures (Soleimani and Zulkifli, 2010). Commercial breeds such as fast growing broilers and high production egg layers are more sensitive to heat stress (Yunis and Cahaner, 1999; Soleimani and Zulkifli, 2010). The Fayoumi birds used in our present study were originally imported from Egypt to the United States in 1954 due to their disease resistance (Deeb and Lamont, 2002) and had evolved with a

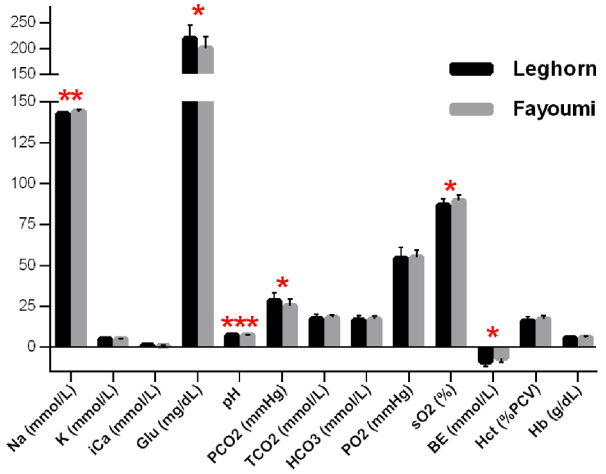

Figure 5. Blood parameter differences between genetic lines at the chronic heat stress and NDV infection stage. Blood parameter differences between heat stress and NDV treated genetic lines at the chronic heat 2 stage. *indicated significant pairwise difference between lines ($P < 0.05$); **indicated $P < 0.01$, and ***indicated $P < 0.001$.

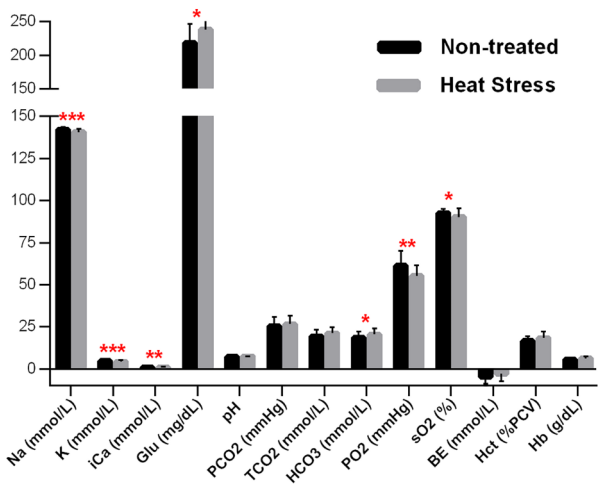

Figure 6. Phenotypic responses to heat stress at the acute heat stage in the Leghorn line. Blood parameter differences between acute heat stressed and non-treated birds in the Leghorn line. *indicated significant pairwise difference ($P < 0.05$); **indicated $P < 0.01$, and ***indicated $P < 0.001$.

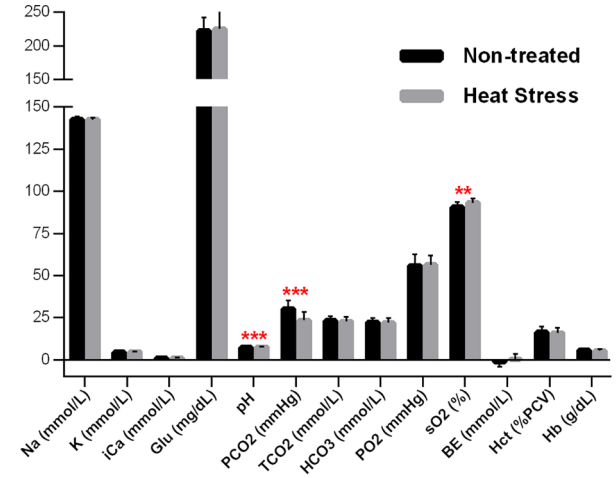

Figure 7. Phenotypic responses to heat stress at the acute heat stage in the Fayoumi line. Blood parameter differences between acute heat stressed and non-treated birds in the Fayoumi line. **indicated significant pairwise difference ($P < 0.01$); ***indicated $P < 0.001$.

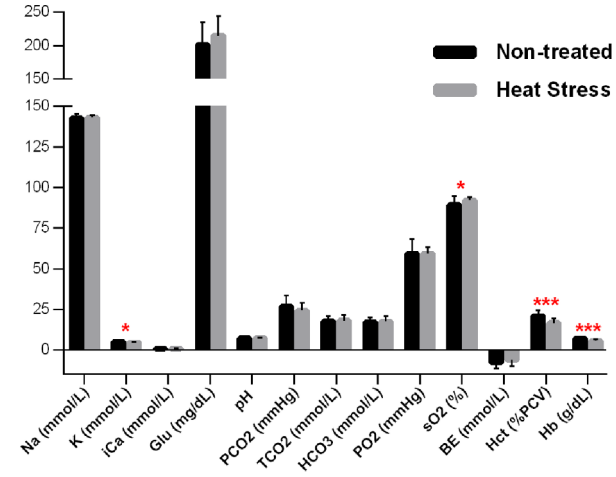

Figure 8. Phenotypic responses to heat stress at chronic heat 1 stage in the Leghorn line. Blood parameter differences between chronic heat stressed and non-treated birds in the Leghorn line at the CH1 stage. *indicated significant pairwise difference ($P < 0.05$); ***indicated $P < 0.001$.

genetic adaptation to high ambient temperatures (Van Goor et al., 2017). Heat treatment had less impact on Fayoumi birds in a genotype-environment interaction study that revealed significant negative impact of heat on commercial broilers (Essam et al., 2007).

Breed, age, management, and nutritional factors have significant influences on blood chemistry measurements in chickens. Previously established reference ranges for blood chemistry parameters by i-STAT in broiler breeder hens (Martin et al., 2010) and in pullet and laying hens (Schaal et al., 2016) suggested that i-STAT can serve as a reliable tool to evaluate metabolic disturbance during the heat stress in chickens.

There were physiological differences observed between Fayoumi and Leghorn lines in blood chemistries under the normal ambient temperature at the three data collection time points (Figures 1 to 3), which could be due do genetic differences between the two lines. Heat stress treatment expanded the differences between these two genetic lines at the AH stage (Figure 4), and adapted to heat environment after one week at the CH1 stage without significant differences (data not shown), there were then a number of different parameters with both heat stress and NDV inoculation three days later (Figure 5). We assume that Fayoumi is more resilient to heat stress than the Leghorn line as the Fayoumi originated from Egypt, a hot climate region. Additional study in our group from another experiment in a Hy-Line Brown population with the same experiment design revealed a significantly positive correlation between these blood parameters before the heat treatment and growth rate with heat stress

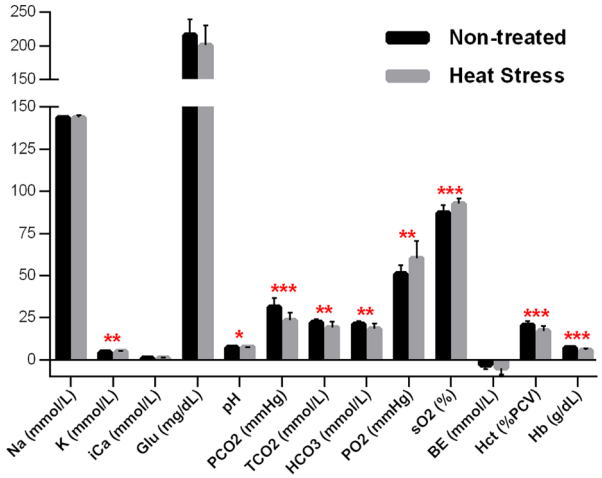

Figure 9. Phenotypic responses to heat stress at chronic heat 1 stage in the Fayoumi line. Blood parameter differences between chronic heat stressed and non-treated birds in the Fayoumi line at the CH1 stage. *indicated significant pairwise difference ($P < 0.05$); **indicated $P < 0.01$, and ***indicated $P < 0.001$.

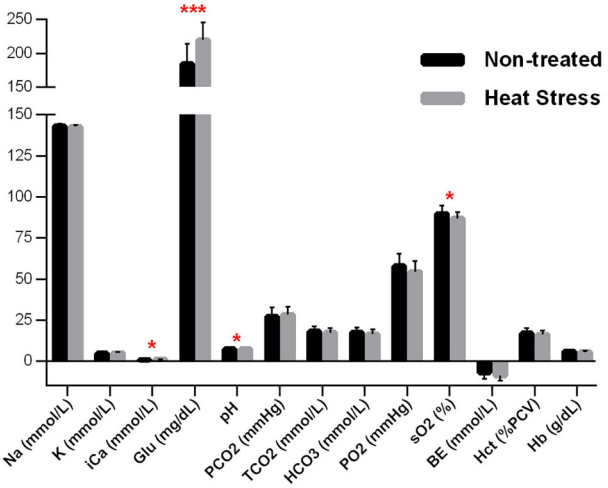

Figure 10. Phenotypic responses to heat stress at chronic heat 2 stage in the Leghorn line. Blood parameter differences between chronic heat stressed and non-treated birds in the Leghorn line at the CH2 stage with NDV infection. *indicated significant pairwise difference ($P < 0.05$); ***indicated $P < 0.001$.

(data not published). Heat treatment resulted in more differences between two genetic lines at the AH stage than the chronic stages, which suggested that these two lines had distinct responses in the course of heat stress. Readouts of blood parameters from Fayoumis could be associated with heat tolerance in chickens.

Within each genetic line between the heat treated and non-treated groups, Leghorn birds had much more rapid responses than Fayoumis at the AH Stage (7 incidences vs. 3 incidences). At the CH1 stage, the opposite situation was observed in that Leghorns had less responses than Fayoumi birds (4 vs. 9). At the CH2 stage with NDV infection, a similar number of parameters were significantly changed in the two lines (4 vs. 5). Most of blood parameters were significantly changed in the opposite directions with the heat stress between

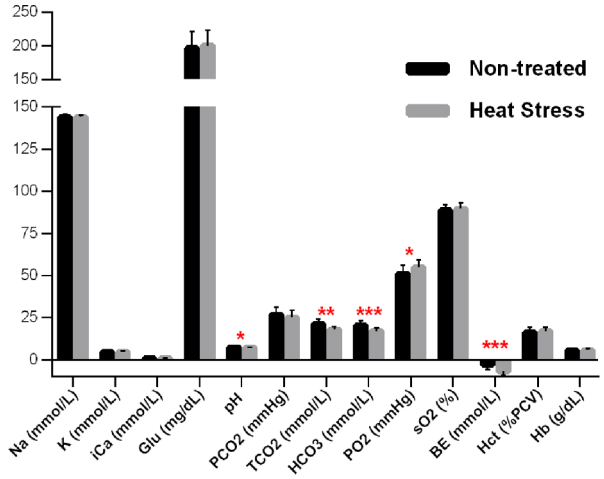

Figure 11. Phenotypic responses to heat stress at chronic heat 2 stage in the Fayoumi line. Blood parameter differences between chronic heat stressed and non-treated birds in the Leghorn line at the CH1 stage with NDV infection. *indicated significant pairwise difference ($P < 0.05$); **indicated $P < 0.01$, and ***indicated $P < 0.001$.

the two genetic lines or significantly changed in only one line (Table 2).

As a heat resilient chicken line, we hypothesize that Fayoumi birds are more elastic in regulating their physiological response to adapt to heat stress, as indicated from changes in their measured blood parameters, which allowed them to suffer less from the heat stress and maintain their homeostasis. Details of these parameters related to physiological response to heat stress are discussed as follows.

Electrolytes/Chemistries

With the heat stress, the balance of blood electrolytes can be disrupted due to large amounts of water intake and disordered homeostasis (Ait-Boulahsen et al., 1995). Many researchers have reported that electrolyte supplements can help birds to alleviate the effects of heat stress (Sahin et al., 2009; Majekodunmi et al., 2013). Three electrolytes, Na^+, K^+, and iCa^{2+} were measured in this study. With the heat treatment, significant loss of Na^+ and K^+ at the AH stage and even less K^+ at the CH1 stage were observed in Leghorn birds (Supplementary Figure S1 to S2) in other reports (Olanrewaju et al., 2006; Olanrewaju et al., 2007). On the other hand, within the Fayoumi line, electrolyte levels in the blood were maintained very well at the AH and CH2 stage, except a rise of K^+ observed at the CH1 stage in heat stressed Fayoumi (Supplementary Figure S3). During heat stress, losing electrolyte balance is a major problems in chickens (Borges et al., 2004). The capability to maintain electrolyte balance is a key factor in maintaining a higher performance (Gamba et al., 2015). Supplementation with electrolytes such as Na^+ and K^+ is one of approaches used to alleviate the impact of heat stress on the performance of chickens (Ait-Boulahsen et al., 1995; Ahmad and Sarwar, 2006).

Table 2. Differences in blood parameters within genetic lines between heat stress and non-treated groups.

	Heat stress (HS) vs. non-treated (NT)					
	Fayoumi			Leghorn		
	AH	CH1	CH2	AH	CH1	CH2
Na^+	NS	NS	NS	-	NS	NS
K^+	NS	+	NS	-	-	NS
iCa^{2+}	NS	NS	NS	+	NS	+
Glu	NS	NS	NS	+	NS	+
pH	+	+	-	NS	NS	-
PCO_2	-	-	NS	NS	NS	NS
TCO_2	NS	-	-	NS	NS	NS
HCO_3	NS	-	-	+	NS	NS
BE	NS	NS	-	NS	NS	NS
PO_2	NS	+	+	-	NS	NS
sO_2	+	+	NS	-	+	-
Hct	NS	-	NS	NS	-	NS
Hb	NS	-	NS	NS	-	NS

Note: NS, not significant between heat stress and non-treated groups; -, heat stressed birds lower than the non-treated birds ($P < 0.05$); +, heat stressed birds higher than the non-treated birds ($P < 0.05$).

Comparing with Leghorn birds, Fayoumis were able to keep a better electrolyte balance during the heat stress treatment. Fayoumi birds were able to raise the K^+ level up at the CH1 stage, which could be one of their mechanisms to preserve higher performance and have higher tolerance to heat stress.

Three dynamic forms of calcium exist in the blood, calcium bounded to protein, calcium complexed with organic acid, or ionized calcium (iCa^{2+}) (Odom et al., 1986). Free iCa^{2+} in blood was mostly used for bone mineralization (Sgavioli et al., 2016) or used by the shell gland (Odom et al., 1986). Many studies have reported significant reduction in blood iCa^{2+} during heat stress (Odom et al., 1986), however a few studies also reported that heat stress did not reduce blood iCa^{2+} (Samara et al., 1996). In the current study, iCa^{2+} levels of heat stressed Leghorn birds were significantly elevated in both AH and CH2 stages (Supplementary Figure S4). As Leghorn line was originally composed of US commercial layer, maintaining a higher iCa^{2+} level could be critical for them to have better performance in their later lives. Meanwhile, CH2 stage was a combination of both heat stress and NDV infection. Calcium levels were able to be increased by hepatitis C virus infection in humans (Paracha et al., 2013). Therefore, the elevation of iCa^{2+} could also be due to NDV inoculation at CH2 in our study.

There were no significant differences in glucose levels between the two genetic lines before exposure to heat. Significant glucose differences between the two genetic lines were found at the CH2 stage with heat exposure and NDV infection, in which Fayoumis had lower glucose levels in blood than Leghorns. With heat stress, Leghorn birds significantly increased their glucose levels compared to non-treated birds (Supplementary Figure S5). Blood glucose concentration may be altered due to various stressors including heat stress (Soleimani et al., 2011). It has been reported that heat stress can result in increase of corticosteroids in the blood plasma, which then subsequently causes an increased blood glucose concentration (Ayo et al., 2011). Heat-stressed animals can use glucose as a fuel by increasing hepatic glucose output through glycogenolysis and gluconeogenesis (Rhoads et al., 2013). Fayoumi birds had lower glucose levels than Leghorns with chronic heat stress in the current study. Therefore, we speculate that Fayoumi birds might depress glycogenolysis and gluconeogenesis metabolisms to "wait out" heat stress, which could be a physiologic mechanism to be resilient to heat stress.

Blood Gases

There were seven blood gas parameters in which pH, PCO_2, TCO_2, HCO_3, and BE are closely related with each other and are also associated with blood CO_2; sO_2 is calculated based on PO_2 and both of them are associated with blood oxygen (O_2).

pH, PCO_2, TCO_2, HCO_3, and BE CO_2 related parameters are indicators of the acid-base balance in blood. Maintaining a stable acid-base balance is a beneficial responses for chickens to be tolerant to high ambient temperature (Gamba et al., 2015). PCO_2 is a major component used to evaluate ventilation and respiratory response of the acid-base status (Ardiaca et al., 2013). During heat-stress treatment, birds with more CO_2 loss via the lungs reduced the PCO_2 in the blood (Sandercock et al., 2001; Borges et al., 2003; Ahmad and Sarwar, 2006; Yalcin et al., 2008; Angell and Seymour, 2015), subsequently, the decreased PCO_2 resulted in increased plasma pH with an alkalosis. TCO_2 measures the total carbon dioxide and can be calculated from pH and PCO_2. Both TCO_2 and HCO_3 can be used in the evaluation of the acid-base imbalance (Hamm et al., 2013). As a major metabolic component of the acid-base balance (Ahmad et al., 2008), increased HCO_3 also caused metabolic alkalosis (Ahmad and Sarwar, 2006; Angell and Seymour, 2015). Blood BE is calculated by the combination of pH and HCO_3, and is one of

indicators of non-respiratory metabolic disturbance of the acid-base status (Ardiaca et al., 2013).

Based on readouts of PCO_2 and pH, Fayoumis had significant respiratory alkalosis at both AH and CH1 stages, but not the CH2 stage (Supplementary Figures S6 to S7), while the Leghorn had no significant changes except a decrease of pH at the CH2 stage. Based on readouts of HCO_3 and BE, Fayoumis had significant chronic metabolic acidosis (Supplementary Figures S8 to S9), while Leghorns had significant acute metabolic alkalosis (Supplementary Figures S10). These results were partially consistent (consistent in HCO_3 in the chronic phase, and in the opposite direction in PCO_2) with a previous heat stress study in which naked neck (heat tolerant) and non-naked neck Rhode Island Red layers were used for evaluating blood gas changes with acute (1 hour) and chronic heat stress (8 wk) (Pech-Waffenschmidt et al., 1995). The inconsistency could be due to different experimental designs and different genetic lines used. In summary, heat tolerant birds (Fayoumi) had phenotypes of early respiratory alkalosis and late metabolic acidosis, while heat susceptible birds (Leghorn) had phenotypes of early metabolic alkalosis. At the CH1 stage, Fayoumi birds were able to lower TCO_2 and HCO_3 to compensate the respiratory alkalosis. However, this was accomplished at the CH2 stage by having a lower pH and normal PCO_2. On the other hand, Leghorn birds had metabolic alkalosis at the AH stage, then stopped responding at the CH1 stage without any changes and with a decrease in the pH at the CH2 stage. NDV inoculation could be a contributing factor to late metabolic acidosis in Fayoumies and pH decrement in Leghorns. To our knowledge, this is the first report in which the dynamic signature of blood gas parameters were used to characterize heat tolerant/susceptible birds.

PO_2, sO_2, and Hematology (Hct and Hb) The other two blood gas parameters PO_2 and sO_2 are mostly associated with the blood O_2 levels. PO_2 is a sensor of pulmonary ventilation (Bollen et al., 2005). The sO_2 measures the amount of O_2 binding to Hb. Fayoumi birds demonstrated much stronger physiological function and elasticity with increased sO_2 and PO_2 in responding to heat stress than Leghorns.

Hct is normally used to determine and monitor the fractional volume of red blood cells (Leypoldt et al., 1995), which is one of the key indicators of the body hydration state as well as the blood's capability to transport oxygen (Fair et al., 2007). One of the heat stress symptoms in poultry is the reduction of blood Hct and Hb concentration, which is a consequence of overhydration due to too much water intake (Hilman P.E., 2000; Puvadolpirod and Thaxton, 2000; Zulkifli et al., 2009). During heat stress, when the temperature difference between birds and environment is decreased, passive temperature reduction (radiation, conduction, and convention) cannot be used for birds to lower core body temperature (Freeman et al., 1999). Therefore, heat loss by hyperventilation is facilitated, which results in an increase of water evaporation through respiration, and drinking additional amounts of water is needed to restore the osmotic balance (Ryder et al., 2004). This subsequently caused reduced Hct and Hb due to hematic dilution (Cristina et al., 2004). Results from our current study were mostly consistent with others by having lower levels of Hct and Hb at the CH1 stage in both genetic lines. Hct and Hb levels were similar between non-treated genetic lines across all time points. However, in the AH stage, heat stressed Leghorn birds had significantly higher Hct and Hb levels than heat stressed Fayoumis (Supplementary Figures S11 to S12). It could because that Leghorn birds slightly increased their Hct ($P = 0.072$) and Hb ($P = 0.062$) levels with heat stress due to suffering from dehydration at the AH stage.

In summary, the present study provided the comprehensive profile of biomarker signatures in blood associated with heat tolerance in the two genetically distinct chicken highly inbred lines. The Leghorn line had a significant acute response to heat, while the Fayoumi had limited early response, but more significant chronic responses during the acclimation to the stress. Specifically, during the heat stress, Leghorn birds had significant electrolyte disruption, while Fayoumis had well-balanced electrolytes, even with enhanced potassium. In addition, Fayoumis had significant respiratory alkalosis and chronic metabolic acidosis, while Leghorns had significant acute metabolic alkalosis. Collectively, the above results suggested potential distinct acute responses between Fayoumi and Leghorn is responsible for determining resilient birds. Finally, significant basal level differences between Fayoumi and Leghorn in PO_2, TCO_2, HCO_3, and BE provided potential biomarkers that can be used to genetically improve heat tolerance in poultry. The insights generated from this study have laid a solid foundation in understanding the genetic control of heat tolerance and susceptibility in poultry. Further investigation on the underlying molecular and cellular mechanisms is warranted.

SUPPLEMENTARY DATA

Supplementary data are available at *Poultry Science* online.

ACKNOWLEDGMENTS

The study is supported by US Agency for International Development (USAID) AID-OAA-A-13-00080. This study is made possible by the generous support of the American people through USAID. The contents are the responsibility of the Feed the Future Innovation Lab for Genomics to Improve Poultry and do not necessarily reflect the views of USAID or the United States Government. This project was partially supported by Multistate Research Project NE-1334 (CA-D-ASC-2156-RR), NC1170 (CA-D-ASC-2155-RR), from the USDA Cooperative State Research, Education, and Extension Service.

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